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Talin anchors and nucleates actin filaments at lipid membranes

A direct demonstration

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Platelet talin nucleates actin assembly as we show here directly by using rhodamine–phalloidin labelling of actin filaments. Nucleation by talin still occurs after reconstitution into liposomal bilayers. This is also demonstrated directly after protein–lipid double labelling and light microscopic imaging. Talin, thus, is the first actin binding protein for which anchoring *and* nucleation of actin filament growth at lipid interfaces have been visualized.

Talin; Platelet; Actin nucleation; Lipid binding

1. INTRODUCTION

Actin assembly at membrane interfaces is a major requirement for cell movement. In search of factors which mediate both filament anchoring and nucleation of actin polymerization, the focal contact and leading edge protein, talin [1,2] proved suitable: talin binds actin monomers with low affinity [3,4], nucleates filament formation in a strongly co-operative manner [5,6] and anchors to lipid bilayers by selectively interacting with lipids [6–8]. These findings are the basis of our current working model according to which talin is an integral membrane protein which induces actin filament nucleation on the cytoplasmic side of the plasma membrane [9].

For the first time, we are able to present evidence that talin, in a membrane-bound, reconstituted state, behaves in a similar manner as observed in solution studies.

2. MATERIALS AND METHODS

2.1. *Proteins*

Skeletal muscle, actin, and human platelet, talin, were prepared as described in [5,6]. For all experiments, 5 μM actin was polymerized in F buffer (2 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 100 mM KCl, 0.2 mM DTT, 0.2 mM $CaCl_2$, 0.5 mM ATP) for 2 h in the presence/absence of talin, talin-vesicles or pure vesicles with the addition of anti-oxidants: 100 mM glucose, 100 μg/ml glucose oxidase and 50 μg/ml catalase.

2.2. *Optics*

Before dilution and recording, actin filaments were labelled with rhodamine–phalloidin (Sigma) in a molar ratio of 1.1 for 20 min at room temperature. Images were taken on a Zeiss Axiomat 10 equipped with plan-neofluar × 63 pH2 objective with a numerical aperture of 1.4, and recorded with a sit camera (Hamamatsu C 2400) connected to a video tape super VHS recorder (Panasonic).

2.3. *Vesicles*

Mixed phosphatidylserine/phosphatidylcholine (PS/PC) vesicles (1:1 w/w, 5 mg/ml total) were swollen in the absence or presence of 3.7 μM talin in 20 mM HEPES buffer, pH 7.4, 0.2 mM EGTA, 0.2 mM DTT for 3 h at 42°C [10]. PS/PC vesicles were labelled with 0.092‰ dimyristoyl-phosphatidylethanolamine (DMPE) which was fluorescently marked by Texas red (Molecular Probes). Talin was previously dialyzed against the vesicle buffer. To concentrate the vesicles and to remove unbound protein, the proteo-liposomes were centrifuged at 20,000 × *g* for 20 min at 4°C.

Talin vesicles were resuspended in a small volume of G buffer (2 mM Tris-HCl, pH 8.0, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.2 mM DTT) in the presence of anti-oxidants. Polymerization of actin was started by the addition of F buffer (final volume 0.5 ml).

At 100% recovery of lipids and at 60–70% recovery of protein, the final concentrations (protein/lipid molecular ratio of 1:3,000) in these assays were: 10 mg/ml lipid, 4.4 μM talin, 5 μM actin.

3. RESULTS AND DISCUSSION

The nucleation of polymerizing actin is significantly enhanced in the presence of talin [5]. At steady state, this results in an increase in filament number concentration over filament length. This effect of talin has been visualized by using rhodamine–phalloidin-labelled actin filaments which have been polymerized in the absence or presence of 3.7 μM talin. For better optical quantification, these filaments are viewed after attachment to non-coated glass coverslips (Fig. 1A,B). The average filament length of polymerized actin is around 22 μm, however, this length is reduced to approx. 5 μM in the presence of talin at a given concentration (Fig. 2). Re-

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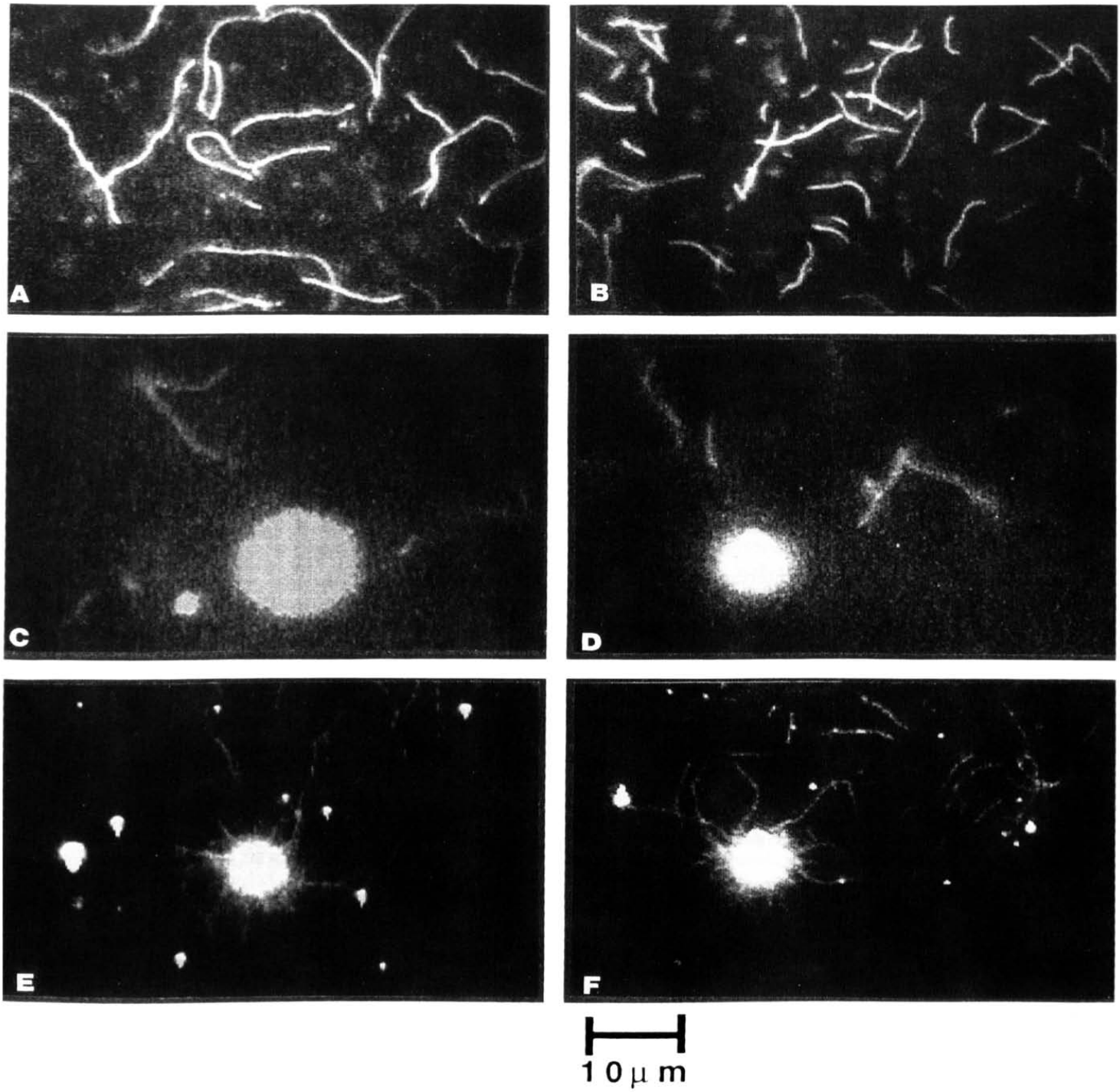

Fig. 1. Fluorescent imaging of 5 μM rhodamine-phalloidin-labelled actin filaments in the absence (A) or presence (B) of 1.25 μM talin. Polymerization of actin in the presence of lipid vesicles (C,D) and in the presence of lipid vesicles with reconstituted talin (protein/lipid ratio of 1:2,000) (E,F). Note: length reduction of phalloidin-stabilized actin filaments due to dilution is negligible on the time scale of observation.

markably, the addition of talin produces a filament population which peaks around a uniform length.

To investigate whether membrane-bound talin is still able to nucleate actin filament formation, talin was reconstituted into mixed PS/PC vesicles of various lipid ratios (Fig. 3). Unbound and stably incorporated proteins were separated into supernatant (S) and vesicular pellet fractions (P) after centrifugation. A maximum of 60–70% protein incorporation was achieved at a PS:PC molar ratio of 1:1 (Fig. 3). To view lipid vesicles and rhodamine-phalloidin-labelled filaments simultaneously, PS/PC vesicles were fluorescently marked with

0.002‰ Texas red, labelled (DMPE) and observed at an emission wavelength of 570 nm and 610 nm, respectively. Vesicles from identical preparations with and without reconstituted talin were incubated with actin, and the formation of filaments was viewed in a free-floating 3D solution in order to avoid an artificial attachment of actin filaments to vesicles near the glass surface. For this reason the coverslips were, in addition, coated with a dimyristoylphosphatidylcholine (DMPC) bilayer prior to use.

In control samples containing vesicles without reconstituted protein, no actin filaments could be found to be associated with vesicles under polymerization conditions (Fig. 1C,D). On the contrary, because of the negative net charge of both filaments and vesicles, actin filaments were rejected from the vesicle surface.

Individual actin filaments could be demonstrated to arise from the vesicle surface when actin is polymerized in the presence of vesicles into which talin was reconstituted (Fig. 1E,F). Since talin is neither a capping nor a severing protein [5], it is assumed that growth occurs at both ends of the filament but may be restricted in regions proximal to the lipid interface. Because of the weak binding (K_d approx. 0.3 μM) of talin to actin [4], filament release from the vesicle after nucleation most probably occurs. A precise optical length determination in a free-floating solution is difficult to establish. However, fluorescent imaging clearly reveals a reduction of the overall filament length in the bulk solution. This is consistent with the release of short filaments from the vesicle surface since no free talin is available to induce this effect.

In conclusion, talin, when reconstituted into lipid vesicles, is able to anchor actin filaments at the lipid interface and to promote growth, as is reflected by an increase in filament number and the overall reduction of actin filament length in these assays.

These observations are in agreement with micro-rheologic studies. Frequency-dependent measurements show that talin, in a concentration-dependent manner, induces the rapid formation of short filaments, suppresses the internal oscillating mode of actin chain dynamics and thus gives rise to an increase in filament stiffness [11].

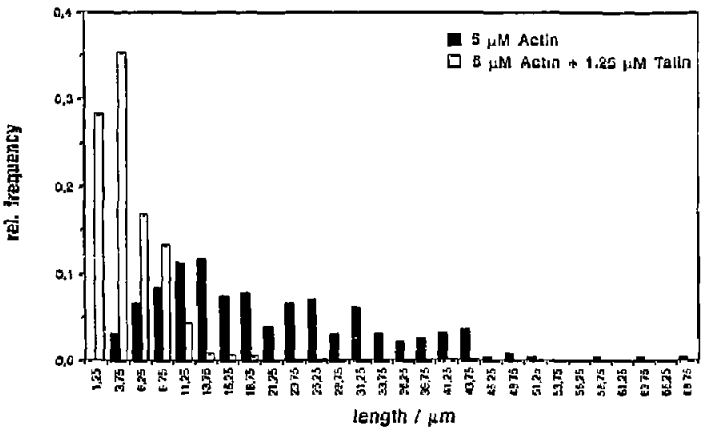

Fig. 2. Length distribution of 386 and 240 individual actin filaments polymerized with (open bars) and without (filled bars) added talin, respectively.

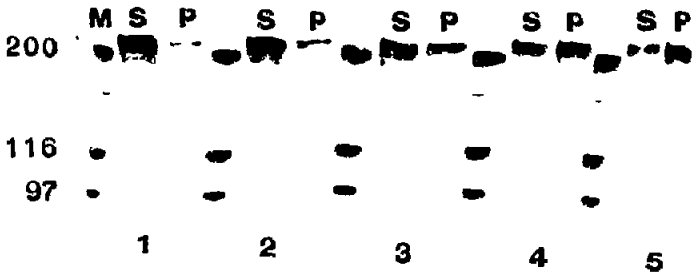

Fig. 3. Reconstitution of talin into mixed PS/PC vesicles. Unbound protein (supernatant, S) is separated from stable vesicle fractions (pellets, P) with increasing phosphatidylserine concentrations. Experiments 1–5 were carried out at a total lipid concentration of 5 mg/ml. M, molecular weight markers in kDa. PS/PC ratios in %: (1) 10/90; (2) 20/80; (3) 30/70; (4) 40/60; (5) 50/50. Quantification by gel scans (not shown) revealed an optimal recovery of talin (70%) in the vesicle fraction at a PS/PC ratio of 1:1.

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